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Comparison of Dermal and Epithelial Approaches to Laser Tissue Soldering for Skin Flap Closure

Donald D. Suh, BS, Ian P. Schwartz, BS, Douglas A. Canning, MD, Howard M. Snyder, MD, Stephen A. Zderic, MD, and Andrew J. Kirsch, MD*

Division of Pediatric Urology, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104

Background and Objective: **Prior studies of laser tissue soldering (LTS) of epithelial skin have shown poor wound strength in the short-term; however, we hypothesize that greater tensile strength and healing properties will result from directing laser energy to the dermal aspect of the skin. The current study compares wound strength and histology in a rat skin flap model of epithelial and dermally applied LTS.**
Study Design/Materials and Methods: **Skin flaps (2.5 × 4 cm) were raised and bisected on the dorsum of Sprague-Dawley rats. The center line of bisection was closed from a dermal approach by LTS (LTS-D, diode laser 15.9 W/cm^2 + Columbia solder), the upper incision by epithelial LTS (LTS-E), and the lower incision by suturing (7-0 Vicryl). Wound skin strips (1–2 mm × 10 mm) were studied immediately (N = 14) and at 3 (N = 57), 7 (N = 31), and 10 (N = 28) days postoperatively and were subjected to tensiometric analysis. Histologic staining with hematoxylin and eosin and Mallory's trichrome methods were used to define wound architecture.**
Results: **No wound dehiscences were noted in any group. Greater immediate tensile strength was noted in wounds closed by LTS-D (521 ± 61 g/cm^2) versus LTS-E (342 ± 65 g/cm^2); however, this difference was not statistically significant (P = .08). By 3 days, both LTS-D (476 ± 55 g/cm^2) and LTS-E (205 ± 37 g/cm^2) maintained their initial strength; however, LTS-D and sutured (436 ± 49 g/cm^2) wounds were stronger (P < .05) than LTS-E. At 7 and 10 days, LTS-D (2,433 ± 346 g/cm^2 and 3,100 ± 390 g/cm^2) showed superior tensile strength (P < .05) compared to both LTS-E (1,542 ± 128 g/cm^2 and 2,081 ± 219 g/cm^2) and suturing (1,342 ± 119 g/cm^2 and 1,661 ± 115 g/cm^2). Histologic analysis of LTS-D wounds at 3 days showed full-thickness tissue apposition, complete epithelialization, and minimal inflammation or thermal injury. At 7 days, solder was present in the wounds. In contrast, LTS-E wounds at 3 days displayed lack of epithelialization secondary to thermal injury and partial-thickness tissue apposition. However by 7 days, epithelialization was complete with moderate scarring, and no solder was seen. Sutured samples appeared similar to LTS-D, except for poorer tissue apposition at the hypodermis.**
Conclusion: **Our results show that skin flap wound healing after dermal LTS is superior to epithelial LTS and emphasizes the**

*Correspondence to: Andrew J. Kirsch, M.D., Georgia Urology, Division of Pediatric Urology, 81 Upper Riverdale Rd., Suite 200, Riverdale, Georgia 30274.

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importance of site specificity in the utilization of this operative technique in reconstructive surgery. Lasers Surg. Med. 22:268–274, 1998.

Key words: dermis; epithelium; solder; wound closure; wound healing

INTRODUCTION

Laser tissue soldering (LTS) has shown great promise in reconstructive surgery by creating a leak-free tissue closure while minimizing the use of suture material and tissue handling. Experimental trials of vas deferens, urethra, microvessel, and nerve repair using LTS clearly have demonstrated improved wound healing and maintenance of tissue architecture and function when compared to conventional methods. Recent clinical trials have involved laser tissue welding for vasectomy reversal [1,2], hypospadias repair [3,4], microvessel anastomosis [5], and bilateral mammaplasty skin wound closure [6]. These studies clearly demonstrate the feasibility of the laser technique; however, its clinical benefit over conventional methods will require further investigation.

Recently, we have demonstrated superior tensile strength and healing characteristics following nearly sutureless skin flap closure using LTS applied to the dermal aspect of the skin [7]. Prior studies of laser welding/soldering on other tissue surfaces such as intestinal mucosa [8] and epithelial skin have not shown adequate early wound strength to allow for primary wound healing. Comparisons of strength relations and healing properties in these tissues have been difficult because of diverse experimental conditions and tissue types. Laser tissue closure, in order to show a benefit over standard suturing, must provide improved tensile strength in the short term while resulting in less scarring later. In the current study of skin flap wound healing, we sought to identify the precise area of the skin (epithelial or dermal surfaces) where LTS would provide the greatest wound strength and optimal healing characteristics.

MATERIALS AND METHODS

Albumin-Based Laser Solder Preparation

The preparation of our solder (human albumin + indocyanine green dye), developed at Columbia University by Bass, Libutti, and Eaton [9], was recently described in detail [3]. Albumin in combination with indocyanine green (ICG) dye may be stored in a freezer (–20°C) for at least 1 year without losing its maximum spectral absorbance at 800 nm [4]. Prior to use, the refrigerated solder should be allowed to equilibrate to room temperature for approximately 30 minutes to allow for more reliable heating upon laser activation. Hyaluronic acid, a viscocity-enhancing agent, was not used in the solder preparation.

Diode Laser System

Treatments were performed with a diode laser module (IRIS OcuLight Diode Laser System™, Iris Medical Instruments, Mountain View, CA) coupled to a rounded quartz silica fiberoptic (600-μm core diameter) housed within a plastic handpiece (IRIS Endoprobe™). The laser system consists of a phased array of gallium-aluminum-arsenide semiconductor diodes and produces an invisible laser beam at approximately 810 nm. A red aiming/pilot beam (650 nm) allows visualization of the spot size of the laser during activation. The spot diameter was approximately 2 mm at a distance of approximately 0.5 cm. The maximum diode power output of the laser module is 3 W. The laser parameters for tissue soldering were power 0.5 W, pulse duration 0.5 second, and pulse interval 0.1 second. The power density (power/area of laser beam spot) was approximately 16 W/cm^2.

Operative Procedure

Adult male rats (weighing 250–375 g) were anesthetized with sodium pentobarbital (25–40 mg/kg IP) and had their back skin shaved. A single dose of oxytetracycline (50 mg, 0.25 ml IM) was given prior to making the incisions. After creation of two 2.5 × 4-cm rectangular skin flaps on the dorsum, each flap was bisected with a 1.5-cm full-thickness incision creating an "E" configuration (Fig. 1). The center line of bisection of the flap was closed by dermal laser tissue soldering (LTS-D) after tacking sutures were placed at both ends of the incision and in the middle to closely oppose the skin edges. The upper bar of the "E" was

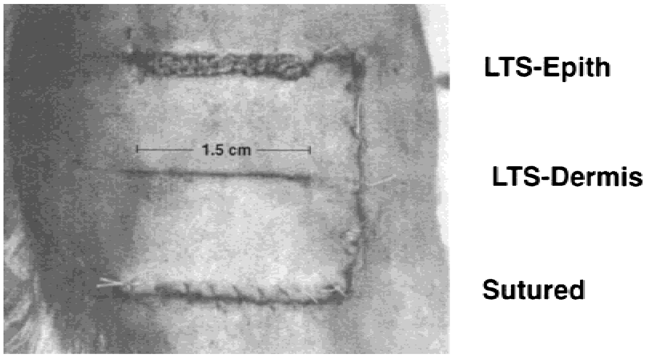

Fig. 1. Rat skin flap model.

closed by epithelial laser tissue soldering (LTS-E). The lower bar of the "E" was closed by running sutures (control, 7-0 Vicryl). The remaining perimeter of the wound was closed with interrupted sutures. Animals were placed in single cages until sacrifice.

Laser Soldering Technique

Our technique has been previously described [3,7,10]. Tissue alignment is mandatory prior to laser activation. Solder that is desiccated between the full thickness of the tissue edges separates the edges further upon laser activation. However, this does not occur when small amounts of solder seep into the incision after being topically applied to appropriately aligned tissue edges. We have found both traction and aligning sutures to aid in tissue approximation of the dermal or epithelial edges. In the current model, two traction sutures (7-0 Vicryl) were placed 2 mm beyond the ends of the 1.5-cm measured incisions. With the traction sutures pulled, the dermal or epithelial edges were aligned. For the dermal-laser-soldered incisions, one additional suture (7-0 Vicryl) was placed in the middle of the incision to further align the skin edges.

Animal Sacrifice and Tissue Preparation

At the time of sacrifice (0, 3, 7, 10 days), animals were injected with a lethal dose of sodium pentobarbital (150 mg/kg). The perimeter of the wound was opened sharply 1–2 cm outside of the rectangular flap, and the undersurface was inspected for abnormalities of wound healing (e.g., adhesions, infection, or seroma formation). Each skin flap was inspected on its exterior and interior surfaces. The lines of incision through the various wound closures were then cut transversely into four to six strips per flap (1–3 mm × 2 cm). Sutures were removed from all strips. Additional strips were placed in formalin prior to paraffin embedding for histological study.

Tensile Strength Measurements

Our tensiometer consisted of a computer-driven motorized slide tray driven at a speed of 2 mm/min using a data acquisition software package (Strawberry Tree™, Inc. Sunnyvale, CA). A force transducer was calibrated to known weights, and a range was established for various force transducers. Analog output was recorded as force versus displacement, and curves were generated on a polygraph recorder (Grass Instruments Co., Quincy, MA). The mean cross-sectional area of each specimen strip was used to calculate peak tension for each specimen. For consistency, skin thickness was determined to be equal (2 mm) for all specimens.

Microscopic Analysis

Five-micron sections of tissue embedded in paraffin wax were sectioned and stained with hematoxylin and eosin and Mallory's trichrome methods. Specimens were examined under a light microscope and photographed.

Statistics

Data for postoperative tensile strength were compared by unpaired Student t-tests. All data were expressed as average ± standard error of the mean. $P < .05$ was considered significant. Qualitative analyses were used for comparing results of gross and histologic data.

RESULTS

Twenty-six full-thickness dorsal skin flaps were raised, bisected, and closed by LTS-E, LTS-D, and running sutures. Animals were sacrificed and evaluated immediately (N = 14), and at 3 (N = 57), 7 (N = 31), and 10 (N = 28) days postoperatively. There were no postoperative complications related to the skin flap. Gross inspection of the flaps at time of sacrifice revealed no evidence of wound dehiscence, seroma, abscess, or hematoma formation. There were minimal-to-no adhesions present between the incision and the underlying muscle fascia in any of the animals.

TABLE 1. Short-Term Tensile Strength (g/cm^2) Measurements

	Sutured	LTS-E	LTS-D
Day 0		342 ± 65*	521 ± 61*
Day 3	436 ± 49	205 ± 37	476 ± 55
Day 7	1,342 ± 119	1,543 ± 128	2,433 ± 346**
Day 10	1,661 ± 115	2,081 ± 219	3,100 ± 390**

*P < .05 compared to sutured wounds.
**P < .05 compared to sutured wounds and LTS-E. Note, at 3 days LTS-D and sutured wounds were significantly stronger (P < .05) than LTS-E.

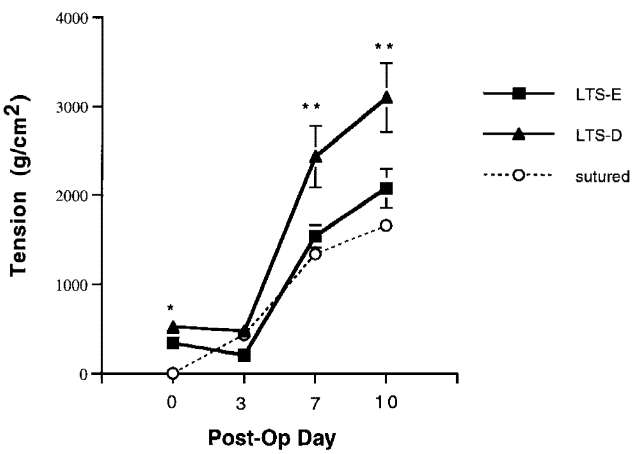

Fig. 2. Short-term wound strength. *P < .05 compared to sutured wounds. **P < .05 compared to both sutured wounds and LTS-E wounds. Note, at 3 days, sutured and LTS-D wounds were significantly stronger (P < .05) than LTS-E wounds.

Samples taken from LTS-E, LTS-D, and sutured incision sites were compared based on wound tensile strength at 0, 3, 7, and 10 days postoperatively (Table 1). Figure 2 shows this data in graph form. Greater immediate tensile strength was noted in wounds closed by LTS-D compared to LTS-E; however, this difference was not statistically significant (P = 0.08). At 3 days, both LTS-D and LTS-E maintained their initial strength (differences in strengths at 0 and 3 days were not statistically significant); however, LTS-D and sutured wounds were significantly stronger (P < .05) than LTS-E wounds. At 7 and 10 days, tensile strength of LTS-D showed superior strength (P < 0.05) compared to both LTS-E and sutured wounds. The differences in tensile strength between LTS-E and sutured wounds were not statistically significant at 7 or 10 days.

Tissue samples taken through the line of incision were examined histologically for evidence of epithelialization, inflammatory response, thermal injury, necrosis, and scarring. At 3 days (Fig. 3), LTS-D wounds showed full-thickness tissue apposition, complete epithelialization, minimal inflammation, and minimal thermal injury to the basal surface striated muscle layer. Under high power, intact muscle bundles were observed in the hypodermis with minimal thermal injury. In contrast, LTS-E wounds at 3 days displayed minimal inflammation, a lack of complete epithelialization secondary to thermal injury localized to the epithelial layer, and only partial-thickness tissue apposition. At 7 days (Fig. 4), no visible solder was seen in LTS-E specimens. Complete epithelialization (with keratinization) was observed to be associated with substantial subepithelial scarring at the site of LTS-E. Qualitatively, a greater degree of early scarring was seen relative to the LTS-D group. Sutured wounds appeared similar to LTS-D wounds showing complete epithelialization; however, there was a lack of apposition of the muscular layer at the hypodermis. In addition, a greater degree of inflammation was associated with sutured closures (Fig. 4).

DISCUSSION

Dermally applied laser soldering led to a wound closure of significantly greater tensile strength immediately after tissue closure, and at 7 and 10 days postoperatively. During normal wound healing, the first 4 to 6 days comprise the early and late inflammatory stages in which the anastomosed area is infiltrated by inflammatory cells and collagenolysis is ongoing. During this time, the laser-activated albumin solder serves as an added protein matrix intermixed with collagen and other extracellular matrix proteins within the lamina propria of the dermis. This interaction allows the underlying tissues to adhere [7] providing the basis for the mechanism of laser soldering. Although the exact biochemical and molecular basis for this interaction is not completely understood, conformational changes in albumin monomers, polymerization, and cross-linking to extracellular matrix proteins and collagen all play important roles in the mechanism of wound healing after LTS [7,11–15].

The fibroplasia stage of wound healing follows the inflammatory stage and marks the time

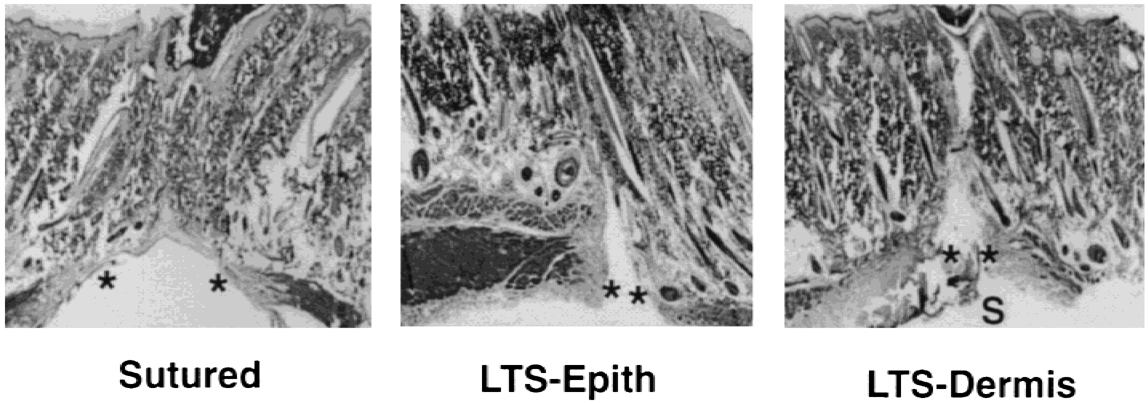

Fig. 3. Histologic specimens at 3 days postoperatively. Note, separation of skeletal muscle within hypodermis in sutured and LTS-E wounds (asterisks mark edges of skeletal muscle in hypodermis). Complete alignment of all tissue layers in LTS-D sample. S, solder. Mallory's trichrome staining, ×50 magnification.

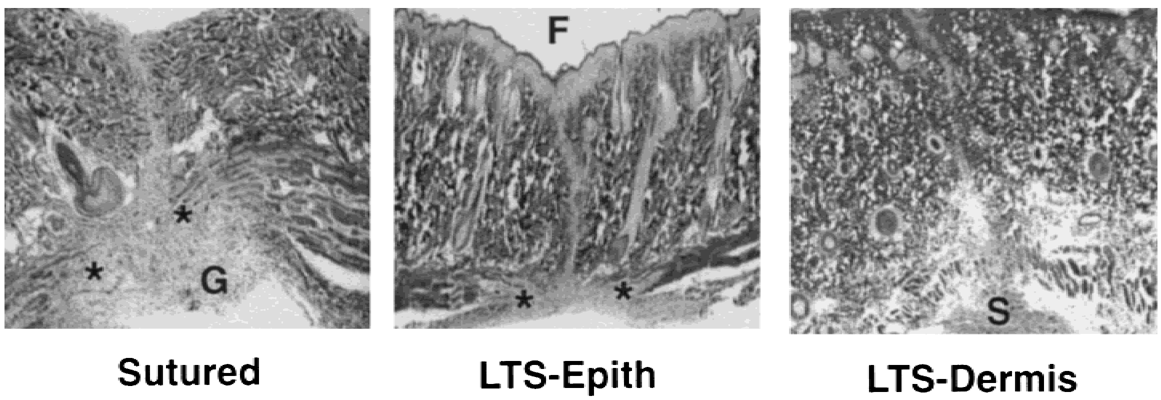

Fig. 4. Histologic specimens at 7 days postoperatively. Asterisks mark edges of skeletal muscle within hypodermis. Note, apposition of all tissue layers in LTS-D sample. G, granuloma; F, furrow (epithelial) secondary to subepithelial injury and wound contracture; S, solder.

when fibroblasts proliferate and produce the extracellular matrix, primarily collagen. During this period, the "scab" of denatured solder acts as a scaffold-like internal and external lattice incorporated into the dermal layers where cellular ingrowth into the solder and normal collagen deposition in the healing wound occurs [7,13]. The dermis is the site of most abundant collagen and actively dividing connective tissue whereas the cornified epithelial layer is a dead cell layer with little or no collagen. This appears to be one of the primary reasons that LTS of the dermal layer results in greater wound strength than LTS of the epithelial layer. Asencio-Arana et al. [16] studied stimulatory effects of a low-power laser beam on fibroblasts and a resultant increase in collagen production per cell; this effect would be greatest in the dermal layer. Eventually this solder is absorbed and most likely does not contribute substantially to wound strength [3]. During the final stage of wound healing, the remodeling stage, collagen matures and forms intermolecular cross-links; there is no net collagen production. There is no evidence to suggest that LTW or LTS provides additional strength over the long term (after 3 weeks).

Close apposition and alignment of wound edges is a crucial factor in achieving successful wound healing in any microsurgical procedure and has measurable effects on tensile strength and scar tissue deposition. Farag et al. [17] emphasized the importance of good tissue apposition

when they found a higher success rate (70%) with enterotomy closure using a Nd:YAG laser in a rat model when less than half of the circumference of bowel was being anastomosed. They observed a 30% success rate when the anastomosis involved greater than half of the circumference. In the current study, we placed tacking sutures to optimally appose skin edges prior to LTS application. During histological analysis we observed that the full thickness of the skin was best aligned in LTS-D skin samples; in LTS-D samples at 3 and 7 days epithelium, dermis, and even the muscularis layer in the hypodermis were apposed. In addition, there appeared to be less scarring present in the wounds closed by LTS-D versus LTS-E or suturing. This may also be related to the closer apposition and better alignment following LTS-D; any degree of gapping may result in healing by second intention and thus more significant scar formation. Interestingly, another method of laser soldering involves laser activation of a column of solder throughout the full thickness of a wound closure; solder is bound to the dermal edges on both sides of the column of solder [14]. This technique may have a potential advantage in the delivery of growth factors incorporated in solder to the full thickness of the wound; however, there may be increased scarring potential secondary to the lack of close apposition of wound edges and healing by second intention.

We suspected that thermal damage to the epithelial layer might play a role in the weaker tensile strength of the LTS-E wounds. Histological analysis showed destruction of the epithelium after LTS-E secondary to thermal injury. While this epithelial layer regenerated by 7 days postoperatively, earlier re-epithelialization is known to provide water-tightness to a wound and is thus desirable in many forms of reconstructive surgery (e.g., urologic, gastrointestinal, vascular).

It was not our intention to directly compare wounds closed by LTS and suturing, but rather to study their wound healing characteristics. Clearly, the strongest tissue closure is by suturing as it represents the tensile strength of the suture material. Sutured wounds (with sutures removed during analysis) in this study showed tensil strength significantly weaker than LTS-D wounds and not significantly different from LTS-E wounds at 7 and 10 days. A potential disadvantage of suturing wounds is the injury caused by needle passage and securing of the knot. This along with foreign body reaction can result in a prolonged inflammatory reaction, granuloma formation, and excessive scarring and adhesion formation. It is important to note that no urethral strictures have been seen following hypospadias repair by LTS in over 50 patients (A.J. Kirsch, personal communication).

Skin flap closure by dermal laser soldering has several distinct advantages. These include minimal tissue handling, maximal tissue alignment, maintenance of luminal continuity, watertight closure, early re-epithelialization, maximal tensile strength during early healing, no foreign body reaction, and minimal scar formation. Our current study has showed that the many benefits of this technology are best achieved when it is specifically applied on the dermal tissue surface.

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